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A Waterhemp (*Amaranthus tuberculatus*) Population Resistant to 2,4-D

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A waterhemp population from a native-grass seed production field in Nebraska was no longer effectively controlled by 2,4-D. Seed was collected from the site, and dose-response studies were conducted to determine if this population was herbicide resistant. In the greenhouse, plants from the putative resistant and a susceptible waterhemp population were treated with 0, 18, 35, 70, 140, 280, 560, 1,120, or 2,240 g ae ha^{-1} 2,4-D. Visual injury estimates (I) were made 28 d after treatment (DAT), and plants were harvested and dry weights (GR) measured. The putative resistant population was approximately 10-fold more resistant to 2,4-D (R:S ratio) than the susceptible population based on both I_{50} (50% visual injury) and GR_{50} (50% reduction in dry weight) values. The R:S ratio increased to 19 and 111 as the data were extrapolated to I_{90} and GR_{90} estimates, respectively. GR_{50} doses of 995 g ha^{-1} for the resistant and 109 g ha^{-1} for the susceptible populations were estimated. A field dose-response study was conducted at the suspected resistant site with 2,4-D doses of 0, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920, and 35,840 g ha^{-1}. At 28 DAT, visual injury estimates were 44% in plots treated with 35,840 g ha^{-1}. Some plants treated with the highest rate recovered and produced seed. Plants from the resistant and susceptible populations were also treated with 0, 9, 18, 35, 70, 140, 280, 560, or 1,120 g ae ha^{-1} dicamba in greenhouse bioassays. The 2,4-D resistant population was threefold less sensitive to dicamba based on I_{50} estimates but less than twofold less sensitive based on GR_{50} estimates. The synthetic auxins are the sixth mechanism-of-action herbicide group to which waterhemp has evolved resistance.

Nomenclature: 2,4-D; dicamba; waterhemp, *Amaranthus tuberculatus* (Moq.) Sauer var. *rudis* (Sauer) Costea and Tardif AMATU.

Key words: Herbicide resistance, auxinic herbicides, growth regulator herbicides.

2,4-D was commercialized as an herbicide for selective weed control in the mid-1940s (Burnside 1996). It is effective at controlling hundreds of broadleaf weed species and is used widely for weed management in fallow, turf, range, pasture, and cereal crop production. Two bacterial genes that confer resistance to 2,4-D have been identified; one has been inserted into soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.), and the second into corn (*Zea mays* L.) (Wright et al. 2010). Soybean, cotton, and corn breeding lines containing these traits are under development for anticipated commercialization. The traits will allow new uses of 2,4-D in all three crops, which will likely increase the volume of 2,4-D applied annually.

Although 2,4-D has been used widely worldwide, only 17 weeds have evolved resistance to this herbicide (Heap 2011). Weeds that have evolved resistance to 2,4-D in pasture and roadside settings include: wild carrot (*Daucus carota* L.) (Stachler et al. 2000; Switzer 1957), Canada thistle [*Cirsium arvense* (L.) Scop.], musk thistle (*Carduus nutans* L.), Italian thistle (*C. pycnocephalus* L.), and tall buttercup (*Ranunculus acris* L.) (Heap 2011). Weeds that have evolved resistance to 2,4-D in wheat (*Triticum aestivum* L.) and other temperate cereal crops include: Indian hedge mustard (*Sisymbrium orientale* Torn.), wild mustard (*Sinapis arvensis* L.), wild radish (*Raphanus raphanistrum* L.), field bindweed (*Convolvulus arvensis* L.), kochia [*Kochia scoparia* (L.) Schrad.], corn poppy (*Papaver rhoeas* L.), scentless chamomile [*Tripleurospermum perforata* (Mérat) M. Lainz], and prickly lettuce (*Lactuca serriola* L.) (Burke et al. 2009; Heap 2011). Weeds that evolved resistance to 2,4-D in rice (*Oryza sativa* L.) or sugarcane (*Saccharum spontaneum* L.) cropping systems include spreading dayflower (*Commelina diffusa* Burm. f.), Sawah flowering rush [*Limnocharis flava* (L.) Buchenau], marshweed (*Limnophila erecta* Benth.), and globe fringebrush [*Fimbristylis miliacea* (L.) Vahl] (Heap 2011). For many of these species, relatively little information is available regarding the magnitude of resistance or the mechanism of resistance. For example, the location of the field bindweed populations reported to be resistant are currently unknown, and the 2,4-D-resistant scentless chamomile populations have been eliminated from the fields where they were originally collected (Heap 2011). Where reported, the magnitude of resistance has varied from 2.5-fold for wild radish (Walsh et al. 2004) to 18-fold for wild mustard (Heap and Morrison 1992) to 25-fold for prickly lettuce (Burke et al. 2009) and 29-fold for globe fringebrush (Watanabe et al. 1997). However, there was a fitness penalty in the absence of 2,4-D selection pressure for globe fringebrush. Without 2,4-D applications for 3 yr, the frequency of 2,4-D resistance in rice fields declined from 86% to less than 2% of individuals (Watanabe et al. 1997). In contrast, MCPA and 2,4-D resistance in musk thistle did not confer an observable fitness penalty (Bonner et al. 1998).

The rate of herbicide-resistance evolution to synthetic auxin herbicides is among the lowest of the herbicide mechanism-of-action groups. Gustafson (2008) estimated a resistance appearance rate of 0.01 species per 1 million hectares sprayed in the United States with a synthetic auxin herbicide, which is one-half the rate of resistance appearance to photosystem II (PSII) inhibitors and one-eighth the rate of resistance appearance to acetolactate synthase (ALS) inhibitors. Numerous reasons for this low rate of resistance have been proposed, including genetic redundancy in plants relative to auxin signaling, the pleiotropic nature of downstream auxin effects, and incomplete resistance where some level of resistance evolves. In a recent review, Jugulam et al. (2011) stated that most cases of synthetic-auxin herbicide resistance are controlled by single genes. Rather than genetic redundancy contributing to resistance, they suggested that the alleles

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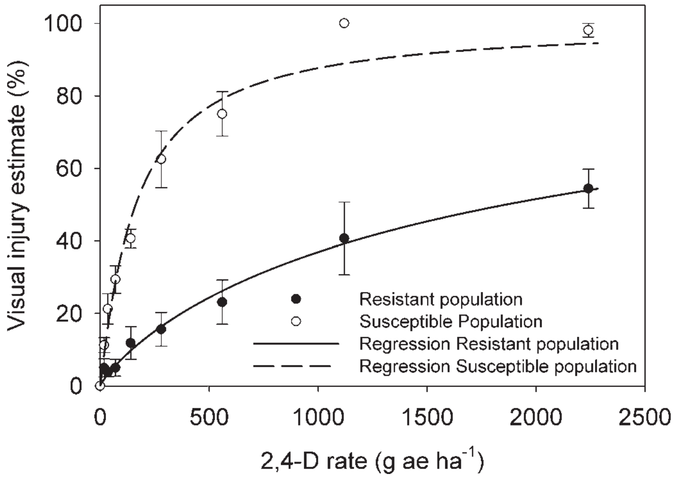

Figure 1. Visual injury estimate as affected by 2,4-D dose for 2,4-D-resistant and -susceptible waterhemp populations at 21 d after treatment in greenhouse bioassays. Regression parameters are provided in Table 1. Data represent the mean of two experiments and four replications per experiment. The error bars represent the standard error for each data point.

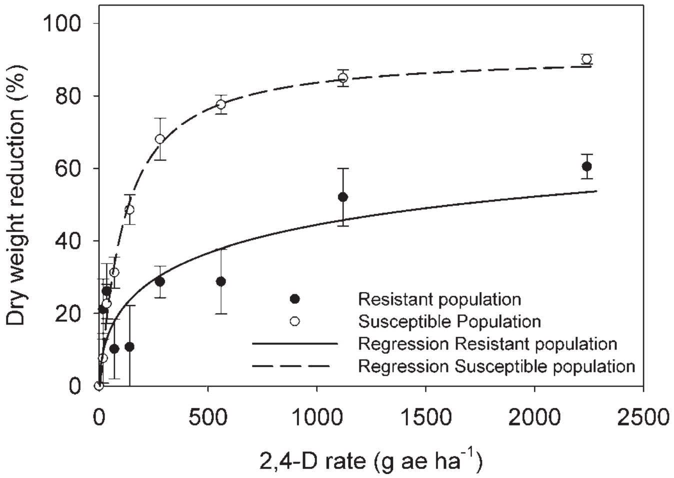

Figure 2. Percent dry weight reduction relative to untreated control as affected by 2,4-D dose at 21 d after treatment of 2,4-D-resistant and -susceptible waterhemp populations in greenhouse bioassays. Regression parameters are given in Table 2. Data represent the mean of two experiments and four replications per experiment. The error bars represent the standard error for each data point.

conferring resistance to synthetic auxins are relatively rare or else they may be lethal (Jugulam et al. 2011). In addition, selection pressure from synthetic auxin herbicides may have been lower than observed for some other herbicide mechanisms of action. For example, synthetic auxins are often applied in combination or in rotation with other herbicides, and the residual life of most synthetic auxin herbicides is relatively short (Jugulam et al. 2011).

Waterhemp has changed from a rarely identified weed in corn and soybean fields 30 yr ago to one of the most problematic weeds in Midwest U.S. crop production (Steckel 2007). The increase in no-tillage crop production, reliance on herbicides for weed management, the extended germination window of waterhemp, and the propensity of waterhemp to evolve resistance to herbicides and then spread the resistant alleles via pollen have contributed to its success (Costea et al. 2005; Tranel and Trucco 2009). Waterhemp has evolved resistance to PSII inhibitors, ALS inhibitors, protoporphyrinogen oxidase (PPO) inhibitors, glyphosate (Heap 2011) and, most recently, to 4-hydroxyphenyl-pyruvate-dioxygenase (HPPD) inhibitors (Hausman et al. 2011; McMullan and Green 2011; Tranel et al. 2011). Waterhemp is a dioecious species and, thus, outcrossing is assured and gene flow among and within populations occurs readily. The accumulation of multiple-resistance genes within populations and even within individual plants is of particular concern. This resistance stacking limits chemical options for managing waterhemp and, where weed management depends primarily on chemical weed control, results in additional selection pressure for the evolution of resistance to the few herbicides that are still effective. In the most severe case to date, individual waterhemp plants were identified with multiple resistance to herbicides from four different mechanism-of-action groups (Tranel et al. 2011).

Soybeans genetically modified to resist 2,4-D (Wright et al. 2010) and dicamba (Behrens et al. 2007) are being anticipated by many farmers as a solution for managing waterhemp and other broadleaf weed populations resistant to other herbicides. However, neither 2,4-D nor dicamba alone are as effective at controlling waterhemp as glyphosate, imazethapyr, and atrazine were when they were first introduced. Many university and industry weed guides estimate waterhemp control with 2,4-D at about 85% (Bernards et al. 2011; Loux et al. 2011; Spandle 2011; Thornsbrough et al. 2010), far less than what is considered acceptable by many farmers. Nevertheless, 2,4-D- and dicamba-resistant crops could provide a new option to aid in waterhemp management.

In the summer of 2009, a grower reported difficulty controlling waterhemp with 2,4-D. The field in question was a warm season grass-production field established in 1996. Since 1996, atrazine, metolachlor, and 2,4-D were applied annually to control annual grasses and broadleaf weeds. The objectives of this research were to: (1) determine if the putative resistant-waterhemp population was indeed resistant

Table 1. Visual injury estimate regression parameters, herbicide doses necessary to achieve 50% injury (I_{50}) (e), I_{80} and I_{90} values, and standard errors (SEs) at 21 d after treatment in greenhouse bioassays for two waterhemp populations.

		Regression parameters						
Population	Herbicide	b	e	SE	I_{80}	SE	I_{90}	SE
Resistant	2,4-D	−0.86	1,864	371	9,300	4,146	23,813	14,504
Susceptible	2,4-D	−1.08	163	20	587	106	1,242	316
R:S	—	—	11.4	—	15.8	—	19.2	—
Resistant	Dicamba	−0.63	150	19	1,446	377	5,435	2,029
Susceptible	Dicamba	−0.64	55	10	387	126	1,211	575
R:S	—	—	2.7	—	3.7	—	4.5	—

Regression parameters were estimated using a four-parameter log-logistic equation (Equation 1), where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point, and e represents the herbicide dose necessary to provide I_{50}.

Table 2. Dry weight reduction regression parameters, herbicide doses necessary to achieve 50% reduction in dry matter (GR_{50}) (e), GR_{80} and GR_{90} values, and standard errors (SEs) at 21 d after treatment in greenhouse bioassays for three waterhemp populations.

		Regression parameters						
Population	Herbicide	b	e	SE	GR_{80}	SE	GR_{90}	SE
Resistant	2,4-D	−0.48	995	407	17,451	19,257	93,238	146,118
Susceptible	2,4-D	−1.08	109	9	396	52	839	155
R:S	2,4-D	—	9	—	44	—	111	—
Resistant	Dicamba	−1.06	54	10	201	62	433	189
Susceptible	Dicamba	−1.14	44	8	150	43	305	175
R:S	Dicamba	—	1.2	—	1.3	—	1.4	—

Regression parameters were estimated using a four-parameter log-logistic equation (Equation 1). For both herbicides, b represents the slope of the line at the inflection point, and e represents the herbicide dose necessary to provide GR_{50}. For 2,4-D, c represents the lower limit (−2.8%, minimum dry weight reduction) and d represents the upper limit (91.5%, maximum dry weight reduction). For dicamba, c represents the lower limit (−3.1, minimum dry weight reduction) and d represents the upper limit (83.7%, maximum dry weight reduction).

to 2,4-D, and (2) determine if the putative-resistant waterhemp population was also resistant to dicamba.

Materials and Methods

In October 2010, seed was collected from a waterhemp population suspected to be resistant to 2,4-D from a native-grass [little bluestem (*Schizachyrium scoparium* [Michx.] Nash 'Camper')] field in southeast Nebraska. Seed from a putative-susceptible waterhemp population was collected from a soybean field near Auburn, NE, in October 2010. Each population sample was a composite of 40 or more plants. Waterhemp seed was cleaned and then stored at 4 C.

Dose-response experiments were conducted in greenhouses located on the East Campus of the University of Nebraska–Lincoln in Lincoln, NE. Sodium halide lamps provided supplemental lighting to ensure a 15-h photoperiod. Daytime temperatures were 24 ± 2 C, and nighttime temperatures were 19 ± 3 C. For the experiments reported in this paper, uniform germination was achieved by placing waterhemp seed on moist filter paper in Petri dishes placed in re-sealable zipper bags and warming them in a dark oven at 35 C for 48 to 72 h. Two healthy seedlings were transplanted into potting mix (Berger BM1 All-Purpose Mix, Berger Peat Moss Ltd., Saint-Modeste, Quebec, Canada) in 10 by 10 by 12.5 cm black plastic pots. Pots were covered with a transparent plastic film for 5 to 7 d after transplanting to minimize desiccation of the seedlings. Plants were watered regularly after the film was removed. Seedlings were thinned to one plant per pot prior to treatment. Treatments were applied to 8- to 12-cm tall plants. Visual injury estimates were made 7, 14, and 21 DAT based on growth suppression and epinastic effects compared to the untreated control plants on a scale of 0 (no injury) to 100 (dead plants). At 21 DAT, plants were cut at the soil surface and shoots were dried for 2 d in a forced air dryer at 65 C, then dry weight was measured for each individual plant.

The experiments were arranged in a randomized complete block design. Each experiment was repeated in time. Four replications were treated with herbicide in each experimental run. At least 10 representative plants of each biotype were harvested prior to treatment in each experiment to provide an average starting dry weight. The first experiment measured responses of the resistant and susceptible populations to nine doses of 2,4-D (Lo-Vol 4, Tenkōz Inc, Alpharetta, GA 30202): 0, 18, 35, 70, 140, 280, 560, 1,120, or 2,240 g ae ha^{-1}. The second experiment measured the responses of the same populations to nine doses of dicamba (Clarity herbicide, BASF Corporation, Research Triangle Park, NC 27709): 0, 9, 18, 35, 70, 140, 280, 560,

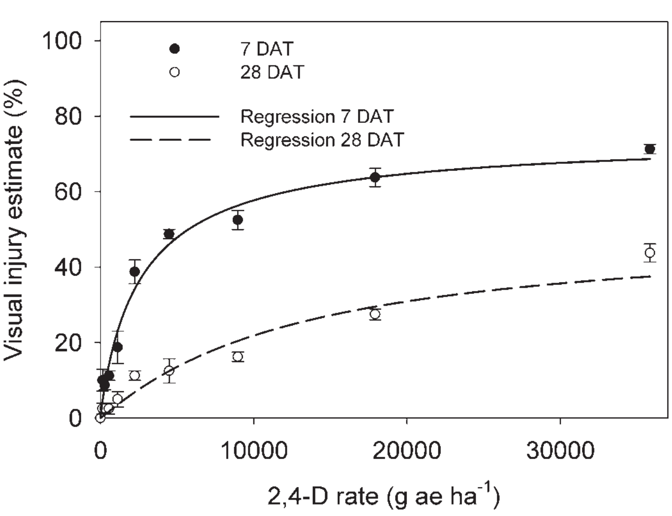

Figure 3. Visual injury estimate as affected by 2,4-D dose at 7 and 28 d after treatment of the 2,4-D resistant population in field bioassays. Regression parameters are given in Table 3. Data represent the mean of four replications. The error bars represent the standard error for each data point.

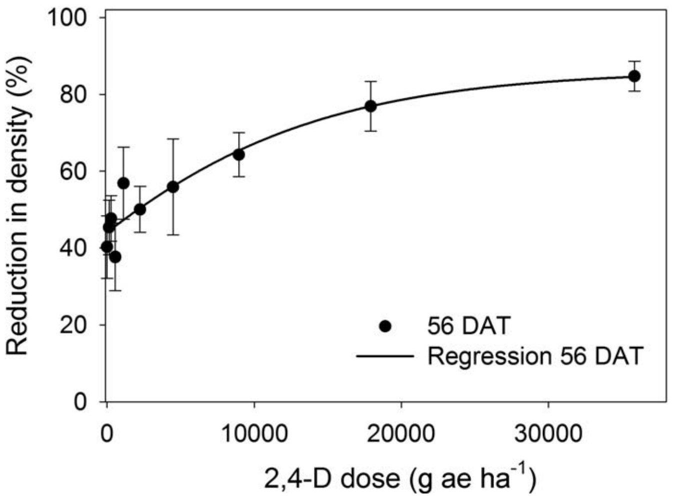

Figure 4. Percent reduction in plant density as effected by 2,4-D dose at 56 d after treatment of the 2,4-D resistant population in field bioassays. Data represent the mean of four replications. The error bars represent the standard error for each data point. Data were fit to a three-parameter Gompertz equation, y = a*exp (−exp[−(x−x0)/b]), where a = 86.2 (standard error [SE] 6.6), b = 10,122 (SE 3,690), and x0 = −4037 (SE 1,460). The R^2 of the fitted line was 0.92.

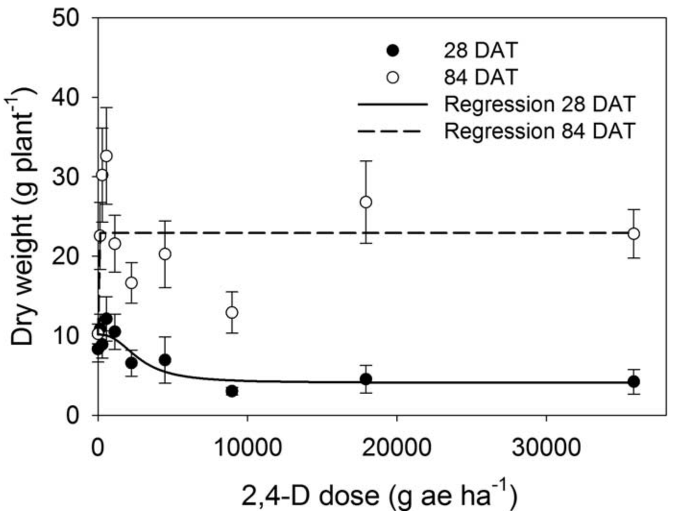

Figure 5. Individual plant dry weight (g plant^{-1}) as affected by 2,4-D dose at 28 and 84 d after treatment (DAT) of the 2,4-D resistant population in field bioassays. Data represent the mean of four replications, two plants per replication at 28 DAT, and five plants per replication at 84 DAT. The error bars represent the standard error for each data point. Regression parameters are given in Table 4.

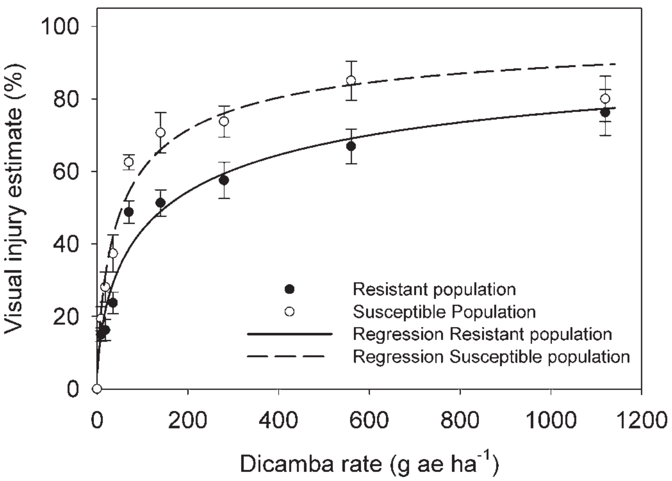

Figure 6. Visual injury estimate as affected by dicamba dose for 2,4-D-resistant and -susceptible waterhemp populations at 21 d after treatment. Regression parameters are provided in Table 1. Data represent the mean of two experiments and four replications per experiment. The error bars represent the standard error for each data point.

1,120 g ae ha^{-1}. Treatments were prepared in distilled water applied in a single-tip chamber sprayer (DeVries Manufacturing Corp, Hollandale, MN 56045) using an 8001E nozzle (Spraying Systems Co., Wheaton, IL 60187) calibrated to deliver 190 L ha^{-1} carrier volume at a pressure of 207 kPa.

A field experiment was established at the site where the resistant population was collected in June 2011. The experiment was arranged in a randomized complete block design with four replications. The plot size was 3 m by 10 m, but only the middle 1.5 m of each plot was treated. Treatments were applied using a backpack sprayer calibrated to deliver 140 L ha^{-1} using four Turbo TeeJet 110015 (Spraying Systems Co.) nozzles spaced 38 cm apart. Treatment solutions were prepared in water, and all treatments contained 0.25% nonionic surfactant (Preference, Winfield Solutions, St. Paul, MN 55164). The 2,4-D doses applied were 0, 140, 280, 560, 1,120, 2,240, 4,480, 8,960, 17,920, and 35,840 g ha^{-1}. At the time of treatment, waterhemp plants ranged from three- to eight-leaf (3 to 27 cm), and the majority of the plants were approximately 15 cm tall. Three 0.25 m^2 quadrats were established along the central axis of each plot, and initial densities were taken. Visual injury estimates were made 7, 14, 21, and 28 DAT. Plant density was taken at 28 and 56 DAT in each of the quadrats. At 28 DAT two plants and at 84 DAT five plants that were representative for size were harvested from each plot, dried, and dry weights were recorded.

Visual injury estimate and greenhouse bioassay dry weight data were analyzed using a nonlinear regression model with the *drc* (drc 1.2, Christian Ritz and Jens Strebig, R2.5, Kurt Hornik, online) package in R (R statistical software, R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) (Knezevic et al. 2007). Dose-response models were constructed using a four-parameter log-logistic equation (Equation 1).

$$y = c + \frac{d - c}{1 + \exp[b(\log\ x - \log\ e)]} \qquad [1]$$

In this model, y is either the % reduction in dry weight or the visual injury estimate, b is the slope at the inflection point, c is the lower limit of the model, d is the upper limit, and e is the GR_{50}. The herbicide dose required to achieve 50, 80, and 90% visual injury or reduction in dry weight was calculated for both herbicides for both populations using the log-logistic models fitted to the data. The R:S ratios were calculated by dividing the GR_{50} of the resistant population by the GR_{50} value of the susceptible population. Standard error bars shown in the figures were calculated for each treatment using mean and standard error functions in SigmaPlot 12.2 (Systat Software, Inc., San Jose, CA). Data for the percent reduction in plant density as affected by 2,4-D dose were fit to a three-parameter Gompertz equation, and data for the change in individual plant weight as affected by 2,4-D dose were fit to a

Table 3. Visual injury estimate regression parameters, herbicide doses (g ae 2,4-D ha^{-1}) necessary to achieve 50% injury (I_{50})(e), I_{80} and I_{90} values, and standard errors (SEs) at 7 and 28 d after treatment (DAT) in field bioassays where the 2,4-D resistant waterhemp population was treated.

	Regression parameters								
Evaluation	c	d	b	e	SE	I_{80}	SE	I_{90}	SE
7 DAT	0	75	−0.92	2,703	212	12,235	1,640	29,593	5,456
28 DAT	0	50	−1.06	12,684	1,190	47,173	8,604	101,712	26,890

Regression parameters were estimated using a four-parameter log-logistic equation (Equation 1), where c represents the lower limit (0 = no injury), d represents the upper limit (100 = plant death), b represents the slope of the line at the inflection point.

Table 4. Individual plant dry weight (g plant^{-1}) estimate regression parameters at 28 and 84 d after treatment (DAT) in field bioassays where the 2,4-D resistant waterhemp population was treated.

	Regression parameters				
Evaluation	c	d	b	e	R^2
28 DAT	4.1	10.1	−2.51	2,761	0.81
84 DAT	10.2	22.9	1.77	2.6×10^{-15}	0.32

Regression parameters were estimated using a four-parameter nonlinear regression model, $y = c + (d-c)/[1 + (x/e)^b]$, where c represents the lower limit, d represents the upper limit, e represents the dose at the inflection point, and b is the Hill slope or slope factor.

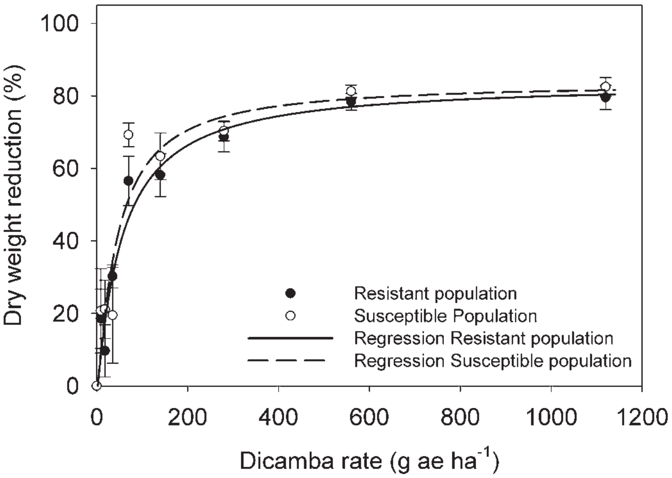

Figure 7. Percent dry weight reduction relative to untreated control as affected by dicamba dose at 28 d after treatment of 2,4-D-resistant and -susceptible waterhemp populations in greenhouse bioassays. Regression parameters are given in Table 2. Data represent the mean of two experiments and four replications per experiment. The error bars represent the standard error for each data point.

four-parameter logistic equation. Both curves were fit in SigmaPlot 12.2.

Results and Discussion

The resistant population was approximately 10-fold more resistant to 2,4-D relative to the susceptible population based on both I_{50} (Figure 1; Table 1) and GR_{50} (Figure 2; Table 1) values. Visual injury estimates for the susceptible population exceeded 98% at 2,4-D doses of 1,120 g ha^{-1} and greater. In contrast, the maximum 2,4-D dose applied (2,240 g ha^{-1}) was not adequate to kill the resistant population. At this highest dose, most plants were stunted and showed epinasty but continued to grow and produced new, normal tissue by the time the plants were harvested. The 2,4-D sensitivity of the susceptible population was compared to that of 41 other waterhemp populations collected in Nebraska and was representative (data not reported). In this single dose screening, average control ranged from 46 to 54% (unpublished data).

The dose response models can be used to calculate the doses necessary to achieve 90% plant injury based on visual estimates and 90% reduction in dry matter accumulation for both the resistant and susceptible populations. However, these calculations are predictions for the resistant population because the highest dose was not sufficient to provide that 90% level of control (Table 1). The R:S ratios calculated for I_{90} and GR_{90} were 19 and 111, respectively. For the resistant population, an estimated dose of 23,800 g ha^{-1} would be predicted to result in 90% visual injury estimates, and a dose of 93,000 g ha^{-1} would be predicted to reduce dry weight 90% relative to an untreated control.

A study was conducted in 2011 at the site where seed from the resistant plants had been collected to quantify response to 2,4-D dose of the population under field conditions. Plots treated with 2,4-D doses of 35,840 g ha^{-1} resulted in 71% visual injury estimates at 7 DAT, but by 28 DAT, plants were recovering and the visual injury estimates were only 44% (Figure 3; Table 3). Increasing 2,4-D dose did affect the density of plants (Figure 4). When compared with plant density at the time of treatment, densities declined in all treatments by 56 DAT. However, as 2,4-D dose increased, the number of plants present declined and reached 85% reduction at the highest dose applied. Although initial injury and mortality were greater as 2,4-D dose increased, plants in each of the treatments recovered (Figure 5). At 28 DAT, dry weight values on a per plant basis decreased as 2,4-D dose increased, but by 84 DAT, 2,4-D dose did not affect individual plant weight (Figure 5). Plants treated with all doses became healthy enough to produce seed (personal observation). The inability to completely control this population at rates 35,840 g ha^{-1} (32 times the highest single application dose labeled for corn) validates the greenhouse data that estimated doses in excess of 23,000 g ha^{-1} for 90% visual injury. Based on its 10-fold decrease in 2,4-D sensitivity in the greenhouse (Table 1) and the lack of susceptibility in the field to extremely high doses, we conclude that the resistant population should be classified as resistant to 2,4-D.

Because it is anticipated that dicamba will also be used widely to help manage glyphosate-resistant *Amaranthus* populations, these two populations were also subjected to a dicamba dose-response study. The 2,4-D resistant population was less sensitive to dicamba than was the 2,4-D susceptible population based on visual injury estimates at 21 DAT (Figure 6). There was a 2.7-fold increase in the dicamba dose required to achieve the I_{50}, and a 4.5-fold increase necessary to achieve the I_{90} in the 2,4-D resistant population (Table 1). The difference in plant dry weight between the resistant and susceptible populations was less than the difference in visual injury estimates (Figure 7). The R:S ratio for the GR_{50} and GR_{90} were also lower (1.2 and 1.4, respectively) than the visual injury estimates. A more narrow R:S ratio for dry weight based measurements relative to visual injury estimates is not unexpected. Plants treated with synthetic auxin herbicides like 2,4-D and dicamba often develop thick callous tissue prior to dying, even when there is little production of normal meristem tissue such as leaves and flowers. It is often easier to describe numerically the damage to the growing point and existing tissue with visual injury estimates.

The magnitude of 2,4-D resistance in this waterhemp population is greater than that reported for wild radish (Walsh et al. 2004) and is comparable to that reported for wild mustard (Heap and Morrison 1992) and prickly lettuce (Burke et al. 2009). Few herbicide mechanisms of action are labeled for use in warm season grasses. Where weed control is important, producers have little choice but to apply the same herbicide(s) repeatedly. In this case, more than 10 consecutive years of 2,4-D use resulted in selection of a resistant population. Similarly, use of 2,4-D for 10 years in New Zealand pastures resulted in selection of a 2,4-D resistant musk thistle population (James et al. 1995).

In conclusion, a population of waterhemp demonstrated at least 10-fold resistance to 2,4-D relative to a susceptible population in greenhouse bioassays. Field studies at the infested site found that 2,4-D doses of 35,840 g ha^{-1} were inadequate to provide 50% control of the population at 28 DAT. This represents the first report of an *Amaranthus* species being selected for resistance to a synthetic auxin herbicide and is the sixth herbicide mechanism-of-action group reported to which waterhemp has evolved resistance. The population also showed reduced sensitivity to dicamba. Technologies such as 2,4-D-resistant corn, soybean, and cotton and dicamba-resistant corn, soybean, and cotton are being developed to provide additional tools for managing waterhemp and Palmer amaranth. The fact that resistance to 2,4-D has evolved in at least one waterhemp population should be emphasized to corn, soybean, and cotton producers to show that proper stewardship of these new technologies is critical for maintaining their effectiveness. The commercialization of soybean, cotton, and corn resistant to 2,4-D and dicamba should be accompanied by mandatory stewardship practices that will minimize the selection pressure imposed on other waterhemp populations to evolve resistance to the synthetic auxin herbicides.

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